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Communication to the Editor

Determination of *N*-(3-Ethylsulfonyl-2-pyridinyl)-4,6-dimethoxy-2-pyridineamine in Soil after Treatment with Rimsulfuron

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Abstract: An analytical procedure to detect *N*-(3-ethylsulfonyl-2-pyridinyl)-4,6-dimethoxy-2-pyridineamine, a degradation product deriving from the hydrolysis of rimsulfuron in soil, has been developed. The analytical standard was prepared by basic hydrolysis of rimsulfuron at pH 9 and purification on a silica gel chromatographic column. The compound obtained was stable at high temperature, thus enabling determination by gas chromatographic analysis. Soil samples were extracted with acetonitrile, purified with a SPE C18 cartridge and analysed using both nitrogen phosphorus (NPD) and mass spectrometer detectors. The analytical procedure described proved to be sensitive and reproducible. Recoveries varied from 84 to 90%. The limit of sensitivity was 0·001 mg kg^{-1}.

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1 INTRODUCTION

Rimsulfuron, 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-ethylsulfonyl-2-pyridylsulfonyl)urea, is widely used as a selective post-emergence herbicide in maize crops. The mode of action of rimsulfuron, like other sulfonylureas, is the inhibition of acetolactate synthase (ALS) and branched-chain aminoacid biosynthesis.[1–3] The specific and high activity of rimsulfuron allows treatments at rates as low as 10–30 g ha^{-1}.

Degradation pathways of rimsulfuron in soil and in hydrolytic solutions under laboratory conditions have shown to produce five degradation products derived from cleavage or contraction of the sulfonylurea bridge.[4] The hydrolysis rate was pH-dependent and was also influenced by temperature. Under field conditions rimsulfuron degraded rapidly with a half-life of 5·6 days and the major metabolite was formed by a contraction of the sulfonylurea bridge forming *N*-(3-ethylsulfonyl-2-pyridinyl)-4,6-dimethoxy-2-pyridineamine.

The very low dosages and rapid dissipation of some sulfonylureas, including rimsulfuron, in soil[1,4,5] complicate residue analysis and although trace-level methods for sulfonylurea herbicides have been proposed,[5,8] it is still not easy to conduct routine analyses of rimsulfuron at trace levels. For this reason the present study was aimed at investigating the stability and persistence of *N*-(3-ethylsulfonyl-2-pyridinyl)-4,6-dimethoxy-2-pyridineamine, which seems to be rimsulfuron's most stable degradation product,[4] so as to develop an analytical procedure to detect it in soil at trace levels. The determination of the major metabolite is necessary

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whenever a parent compound degrades in a very short time: for instance, the fungicide benomyl degrades rapidly to carbendazim. For this reason only carbendazim is determined as the residue in soil and crops. A similar situation might be demonstrated for rimsulfuron.

2 EXPERIMENTAL METHODS

2.1 Apparatus

Gas chromatography (GLC) analyses were performed using a Carlo Erba model 8560 chromatograph, fitted with a nitrogen-selective detector (NPD) and an on-column injector. GC/MS analyses were performed using a Varian Star model 3400 chromatograph, fitted with a split-splitless injector. The mass spectrometer was a Varian Saturn II operating in the electron impact mode and in chemical ionization. HPLC analyses were performed using a LDC analytical, Constametric model 4100 solvent delivery system fitted with a Thermo separation product, Spectromonitor model 3200 variable UV wavelength detector and a Rheodyne injection system. NMR spectra were determined in hexadeutero dimethyl sulfoxide (d_6-DMSO) using a Varian NMR spectrometer 300 MHz. IR spectra were determined using a Nicolet 205 FT spectrometer. Solid-phase extractions were performed using Supelco SPE C18 cartridges (3-ml tubes).

2.2 Reagents and materials

The analytical standard of rimsulfuron was provided by Du Pont Agricultural Products, Wilmington DE, USA. The methylene chloride and acetonitrile were Analar (BDH) grade; the water, acetonitrile and acetic acid used for HPLC analyses were Hipersolv (BDH) grade. The methanol was a special reagent for pesticide analysis (BDH).

A loam soil (Fluventic Xerochrept) was used; the major properties of this soil are shown in Table 1.

2.3 Synthesis and identification of *N*-(3-ethylsulfonyl-2-pyridinyl)-4,6-dimethoxy-2-pyridineamine

Rimsulfuron (1 g) was added to a Tris-HCl buffer solution (0·1 M, 300 ml) at pH 9 and stirred for three days. The solution was extracted with dichloromethane (3 × 100 ml) and the organic extract evaporated just to dryness in a rotary evaporator (40°C). The residue was dissolved in dichloromethane and purified by a chromatographic column filled with silica gel. The column was eluted with hexane + ethyl acetate + acetic acid (20 + 80 + 2 by volume). The eluate was evaporated and the weight of the solid residues was nearly 400 mg. Aliquots of this product were dissolved in methanol and its chemical structure was established by GC/MS, NMR and IR analyses.

TABLE 1
Properties of the Soil

Organic matter (%)	*pH*	*Mechanical analyses* (%)			*CEC* (*meq 100 g*$^{-1}$)
		Clay	*Sand*	*Silt*	
1·81	8·05	29·0	35·0	36·0	18

2.4 Soil treatment with rimsulfuron

Air-dried soil (100 g), passed through a 1·0-mm sieve, was treated with a methanolic solution (5 ml) containing 2 μg ml^{-1} of rimsulfuron. The methanol was evaporated and a further 900 g of soil was added. In this way the concentration of rimsulfuron in soil was roughly the same as that used in field treatment conditions (0·01 mg kg^{-1}). The soil was then stirred in a mechanical shaker for three hours in order to attain an even distribution of the herbicide, and then placed into ten aerated containers (100 g each). Samples were kept in a climatic chamber at 25°C. Distilled water was added daily to each sample to maintain soil moisture at approximately 75% of the field moisture holding capacity. A sample for analysis was removed from the chamber at days 2, 4, 8, 16, 32 and 64. Each sample was kept at −20°C until analysis.

To determine the stability of rimsulfuron in soil, air-dried soil (100 g), passed through a 1·0-mm sieve, was treated with a methanolic solution (5 ml) containing 20 μg ml^{-1} of rimsulfuron, then the same procedure was used.

2.5 Stability trials

To determine the stability of rimsulfuron in solvents with different polarities, rimsulfuron (2·5 mg) was dissolved in methanol (100 ml), acetonitrile (100 ml) and dichloromethane (100 ml), then stored in the dark at 25°C. To determine the stability of rimsulfuron at various pH values, rimsulfuron (25 mg) was dissolved in acetonitrile (10 ml) and the solution obtained (1 ml) was added to different buffer solutions (99 ml) and then stored in the dark at 25°C. Both types of solution (10 μl) were analysed after 1, 2, 4, 8, 16 and 32 days. Three 0·1 M buffer solutions were used: acetate buffer pH 4, monobasic-dibasic phosphate buffer pH 7 and Tris-HCl buffer pH 9. The same procedure was used to check the stability of *N*-(3-ethylsulfonyl)-2-pyridinyl-4,6-dimethoxy-2-pyridineamine.

2.6 Recovery trials

Air-dried soil (100 g) passed through a 1·0-mm sieve was treated with a methanolic solution (1 ml) contain-

ing the amount of rimsulfuron or *N*-(3-ethylsulfonyl-2-pyridinyl)-4,6-dimethoxy-2-pyridineamine needed to obtain the experimental fortification level (see Table 2), then extracted, cleaned up and analysed as described in Section 2.7.

2.7 Analytical procedures

Samples from soil treated with rimsulfuron were analysed as follows:

2.7.1 Extraction and clean-up procedure of N-(*3-ethylsulfonyl-2-pyridinyl*)*-4,6-dimethoxy-2-pyridineamine*
Distilled water (20 ml) and acetonitrile (60 ml) were added to soil (100 g). The mixture was stirred for 1 h, centrifuged at 6000 rev min^{-1} for 15 min and the solution filtered. The samples were then washed with the extracting solution (100 ml), and centrifuged again as before.

The liquid phase was evaporated using a rotary evaporator at 40°C. Methanol (1 ml) and distilled water (20 ml) were then added. The mixture was passed through a SPE C18 cartridge previously activated with water (3 ml) and methanol (3 ml) and the eluate discarded. After rinsing with water (20 ml), the column was then eluted with methanol (6 ml) and the eluate collected and evaporated until just dry. The solid residue was dissolved in methanol (0·2 ml) and this solution (1 μl) was injected into the gas chromatograph equipped with NPD detector and into the combined gas chromatograph/mass spectrometer.

2.7.2 Extraction and clean-up procedure of rimsulfuron
Distilled water adjusted to pH 2·5 with *ortho*-phosphoric acid (20 ml) and acetonitrile (60 ml) were added to soil (100 g). The mixture was stirred for 1 h, centrifuged at 6000 rev min^{-1} for 15 min and the solution filtered.

The samples were then washed with the extracting solution (100 ml), and centrifuged again as before. The acetonitrile was evaporated using a rotary evaporator at 40°C and the aqueous solution extracted with dichloromethane (2 × 30 ml). The organic phase was evaporated using a rotary evaporator at 40°C. The solid residue was dissolved in methanol (1 ml) and this solution (10 μl) was injected into the HPLC system.

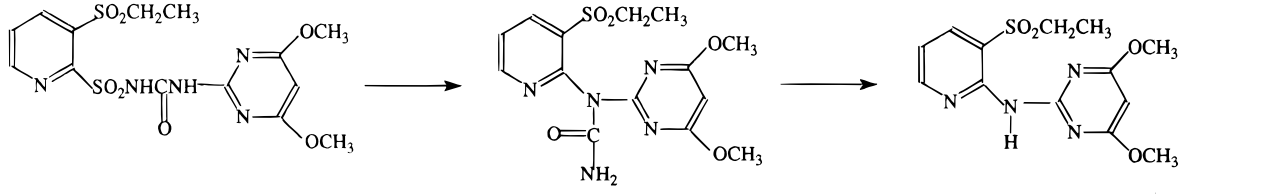
2.7.3 Gas chromatography and GC/MS
The column used for gas chromatographic analyses was a 25 m × 0·32 mm fused silica capillary column coated with 0·25 μm OV17. The temperature was preset to an initial temperature of 50°C (1 min) ramped at 30°C min^{-1} to a final temperature of 270°C (20 min). The carrier gas used was helium (2·5 ml min^{-1}). In these conditions the retention time of *N*-(3-ethylsulfonyl-2-pyridinyl)-4,6-dimethoxy-2-pyridineamine was 20·2 min.

The column used for GC/MS analysis was a 30 m × 0·25 mm fused silica capillary column coated with 0·25 μm DB5. The temperature was preset to an initial temperature of 60°C (1 min) ramped at 15°C min^{-1} to a final temperature of 260°C (5 min). The carrier gas was helium (1 ml min^{-1}), the injector temperature was 270°C, the ionization voltage was 70 eV and the emission current 10 μA. Methane was used as the ionizing agent. In these conditions the retention time of *N*-(3-ethylsulfonyl-2-pyridinyl)-4,6-dimethoxy-2-pyridineamine was 19·4 min.

2.7.4 HPLC
The column used was an octadecyl (C 18) 25 cm × 4·6 mm. The elution solvents used were (A) acetic acid 0·1 M and (B) acetonitrile; the following gradient was used (A : B, linear shifts between all points): 0 min—70 : 30, 5 min—40 : 60, 20 min—40 : 60, 25 min—0 : 100. The flow rate was 1 ml min^{-1}, the detection wavelength was 254 nm. In these conditions the retention time of rimsulfuron was 14·1 min, whilst the retention time of *N*-(3-ethylsulfonyl-2-pyridinyl)-4,6-dimethoxy-2-pyridineamine was 20·3 min.

3 RESULTS AND DISCUSSION

The chemical structure of the product obtained from the hydrolysis of rimsulfuron (compound **3**, Fig. 1) was established by GC/MS, NMR and IR analyses. The mass spectrum obtained by chemical ionization (Fig. 2(a)) shows that the most intense signals were those at *m/e* 325 and at *m/e* 231. The former is the protonated molecular ion of compound **3**, whilst the latter is consistent with the loss of the ethylsulfonyl group from this ion. The signal at *m/e* 216 in the mass spectrum



Rimsulfuron
1

2

N- (3-ethylsulfonyl -2-pyridinyl)-4,6-dimethoxy-2-pyrimidineamine
3

Fig. 1 Transformation of rimsulfuron in soil.

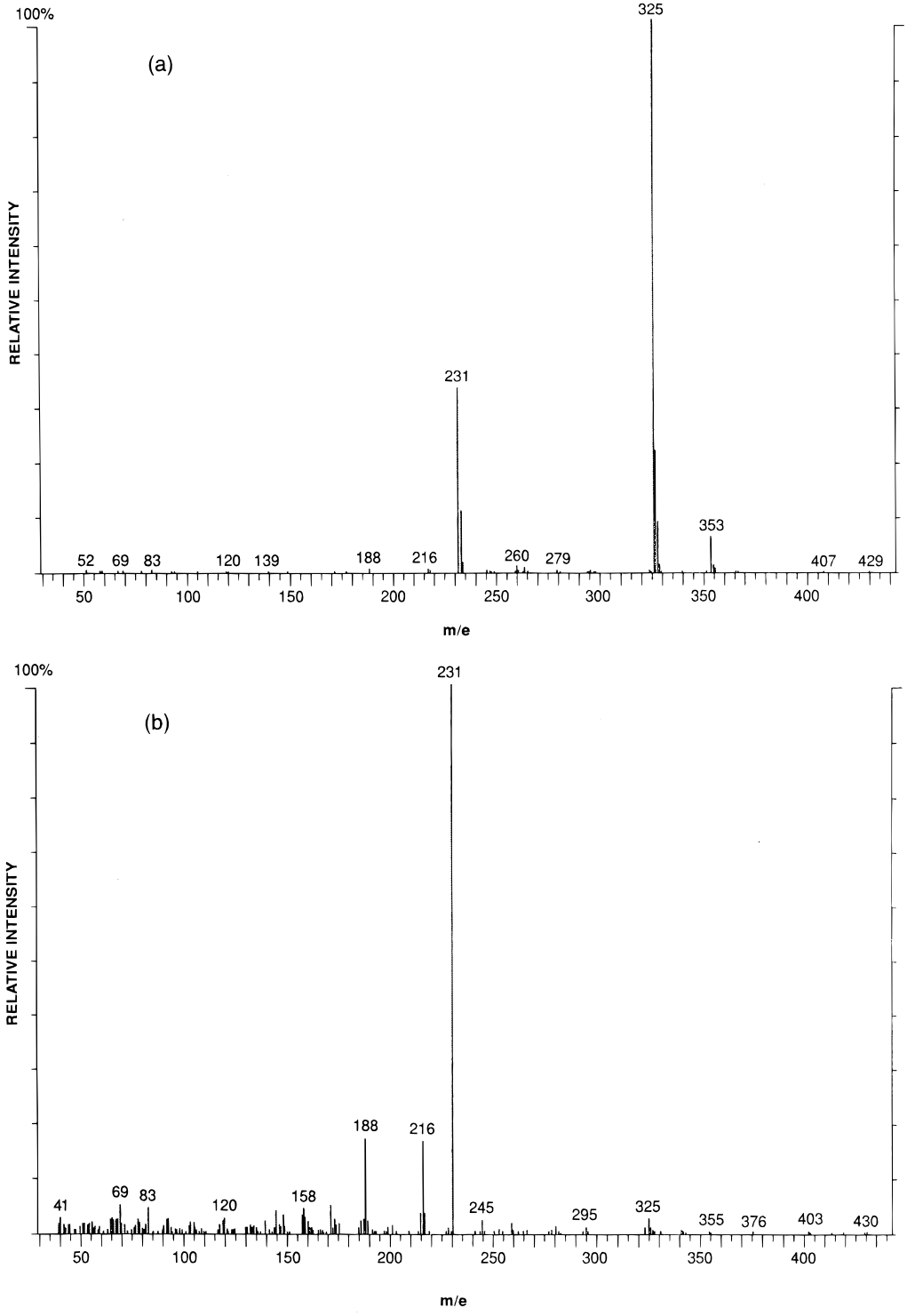


Fig. 2. (a) Chemical ionization and (b) electron impact mass spectra of compound **3**.

obtained by electron ionization (Fig. (2b)) is consistent with the loss of $—CH_3$ from the parent ion 231; the signal at *m/e* 188 was formed by the loss of a methyl group from the parent ion followed by the loss of a C=O group.

In the [^{1}H]NMR spectrum (in d_6-DMSO), the ethyl protons absorbed at δ 1·11 ($—CH_3$) and 3·4 ($—CH_2—$) ppm and the coupling constant was 7·3 Hz. The $—OCH_3$ groups appear as a singlet at δ 3·8 ppm, while the pyrimidinic proton resonated at δ 5·8 ppm. The aromatic pyridinic protons appear as double doublets at δ 7·38 (J = 4·8, 7·8 Hz), 8·23 (J = 1·8, 7·8 Hz) and 8·68 (J = 1·8, 4·8 Hz) and NH as a singlet at δ 9·18 ppm. In the [^{13}C]NMR spectrum, it is noteworthy that the signals at δ 6·9, 53·8, 82·5, 119·6, 139·7 and 153·7 in the Attached Proton Test (APT) corresponded to $—CH_3$ and $=CH—$, whilst those at δ 48·9, 123·4, 150, 158·2 and 171·7 were associated with the $—CH_2—$

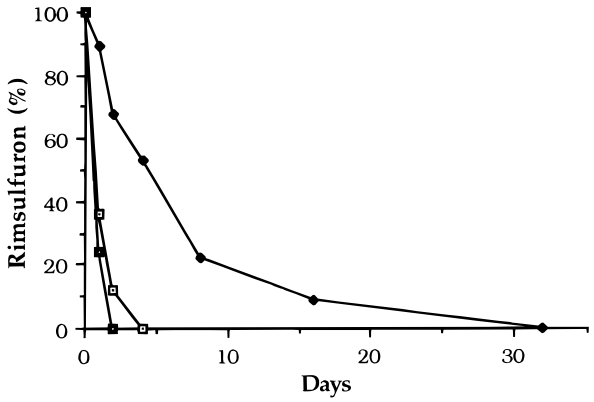


Fig. 3. Degradation of rimsulfuron at (□) pH 4, (◆) 7 and (■) 9.

and quaternary carbon atoms. The assignments were made on the basis of: (a) the reported shifts of substituents, i.e. sulfonyl and amino groups, on aromatic and heteroaromatic compounds; (b) the comparison of chemical shifts of pyridine derivatives and benzensulfonyl compounds. Moreover the IR spectrum gives further proof of the presence of compound **3**, rather than compound **2**, since no C = O bond is present.

Preliminary trials showed that compound **3** was a more stable compound than rimsulfuron, both in soil and in solvents with different polarities (data not shown). In water the compound was stable at pH values between 4 and 9. Analytical standards in the various solvents and at the given pH values showed no changes over the 32-day stability trial period; however, rimsulfuron degraded rapidly at all the pH values tested (Fig. 3). Compound **3** was detected within two days in the soil treated with rimsulfuron at 0·01 mg kg^{-1} under the conditions described in Section 2.4. This compound proved to be stable under gas-chromatographic conditions enabling detection by gas chromatographic and GC/MS analytical methods, as proposed in this paper. In this experiment it was possible to detect compound **3** up to 64 days after treatment (Fig. 4), whilst the rimsulfuron was no longer detectable by HPLC with UV detection, in soil treated at 0·1 mg kg^{-1}, two days after treatment because of its rapid degradation in soil. The limit of sensitivity of the analytical method used to determine the degradation of rimsulfuron was 0·01 mg kg^{-1} and recoveries varied from 76% for 0·01 mg kg^{-1} to 84% for 1 mg kg^{-1} (Table 2).

The efficiency of the analytical procedure described for the analysis of compound **3** residues in soil is indicated by the recoveries of the compound added to untreated samples (Table 2). Recoveries varied from 84% for 0·001 mg kg^{-1} to 90% for 1 mg kg^{-1}. On the basis of the chromatograms of the numerous blanks tested, a value of 0·001 mg kg^{-1} can be fixed as the lower limit of determination. Compared to other methods described in literature[4,6] this analytical procedure would appear to be highly cost-effective and sensitive.

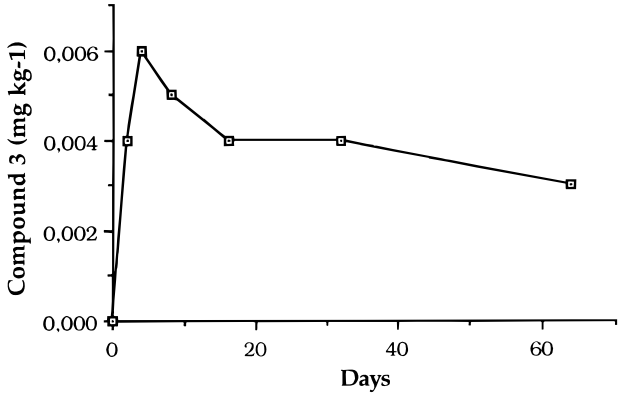


Fig. 4. Compound **3** detected in soil after treatment with rimsulfuron at 0·01 mg kg^{-1}.

TABLE 2
Recoveries of Rimsulfuron and Compound **3** Added to the Soil before Extraction

Rimsulfuron		*Compound 3*	
Added (*mg* kg^{-1})	*Recovery* (%) (±*SD*)[a]	*Added* (*mg* kg^{-1})	*Recovery* (%) (±*SD*)[a]
10	82 (±4·9)	1	90 (±3·1)
1	84 (±4·5)	0·1	85 (±4·0)
0·1	78 (±5·1)	0·01	85 (±3·8)
0·01	76 (±5·0)	0·001	84 (±4·2)

[a] $n = 3$.

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